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Longitudinal assessment of lung function in children with sickle cell disease

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Running head: Lung function in SCD children

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ABSTRACT

Objectives: To prospectively assess longitudinal lung function in children with sickle cell disease (SCD).

Working hypothesis: Lung function in SCD children deteriorates with increasing age and the decline is more marked in younger children who have recently suffered ACS episodes.

Study design: Two prospective longitudinal studies.

Patient-subject selection: Two cohorts of SCD children and age and ethnic matched controls were recruited. Cohort One (47 SCD and 26 controls) had a median age of 8.8 years and follow-up of two years and Cohort Two (45 SCD and 26 controls) a median age of 10.2 years and follow up of ten years.

Methodology: Forced expiratory volume in one second (FEV_1), vital capacity (VC), forced expiratory flow between twenty-five and seventy-five % of VC (FEF_{25-75}), total lung capacity (TLC) and residual volume (RV) were measured on two occasions.

Results: In both groups of SCD children, lung function declined significantly, but in neither control group. ACS episodes were more frequent during the follow up period in Cohort One than Cohort Two ($p<0.0001$). The rate of decline was greater in cohort one than Cohort Two for FEV_1 ($p = 0.008$), VC ($p = 0.001$), FEF_{25-75} ($p = 0.030$), TLC ($p = 0.004$), and RV ($p = 0.043$). In Cohort Two restrictive abnormalities were more common at follow up ($p=0.006$).

Conclusions: Lung function deteriorated with increasing age in SCD children and the rate of decline was greater in younger children in whom ACS episodes were more common.

INTRODUCTION

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide, affecting an estimated 300,000 newborns every year [1]. The majority of children with SCD in developed countries can expect to survive to adulthood [2] but may then suffer severe respiratory morbidity including chronic hypoxia and pulmonary hypertension [3-9]. Lung function abnormalities have been reported even in young SCD children, but there is not a consistent picture, cross-sectional studies highlighting restrictive [10, 11] or obstructive [12-15] abnormalities. One cross-sectional study, however, suggested the occurrence of restrictive abnormalities may increase with increasing age in childhood [11]. More recently, longitudinal studies have been undertaken. In a cohort of 45 children aged between 5 and 18 years measured at baseline and approximately four years later, a predominantly obstructive pattern was reported which increased in prevalence over time; the occurrence of restrictive abnormalities also increased, but to a lesser extent [16]. In contrast, retrospective analysis of a larger cohort of 413 SCD children aged between eight and eighteen years, who were measured on two separate occasions with a variable time to follow-up, demonstrated an increased prevalence of restrictive abnormalities with increasing age [17]. There was a decline in total lung capacity (TLC) of 2.3% per year [17] and a similar decline in forced expiratory volume in one second (FEV1), vital capacity (VC), and forced expiratory flow between 25% and 75% of VC ($FEF_{25-75\%}$). Neither study [16, 17], however, included a control group and thus it is not possible to determine whether the decline in lung function reported was only seen in SCD patients and hence the magnitude of the effect of SCD. In addition, it is important to assess whether any difference was influenced by increasing age.

In adults, acute chest syndrome (ACS) is the commonest cause of death [18, 19] and is associated with more severe restrictive disease [20]. In children, ACS episodes have been associated with reductions in lung function [21, 22]. The impact of ACS episodes on longitudinal changes in lung function, however, has not been investigated [16, 17]. There is evidence that asthma may be a risk factor for ACS episodes [21-28]. Hence, in any subsequent longitudinal study the impact of asthma also needs to be considered.

The aim of this prospective study was to examine the rate of decline in lung function in children with SCD in comparison to age and ethnic matched controls. Two cohorts were studied. Cohort One were younger children who were followed for approximately two years, during an age when ACS episodes are more common [28]. Cohort Two were older and followed for approximately ten years. We included two cohorts as comparison of their results would allow us to test our primary hypothesis that the rate of decline in lung function would be greater in the younger cohort as it would relate to their higher occurrence of ACS episodes. Furthermore, in Cohort Two, we wished additionally to test the hypothesis that any obstructive abnormalities demonstrated at the initial assessment would be replaced by restrictive abnormalities at the subsequent assessment. As asthma may be a risk factor for ACS episodes [21-28] we also wished to determine whether asthma and hence baseline obstructive lung function abnormalities influenced any change in lung function and whether obstructive lung function abnormalities were associated with an increased risk of ACS episodes. Ethnic and age matched controls were recruited for both cohorts to determine whether any decline in lung function was only seen in SCD patients.

MATERIALS AND METHODS

Study design

A prospective longitudinal study of children with SCD (homozygous for sickle cell hemoglobin (HbSS)) of African-Caribbean or West African descent was undertaken. Age and ethnic matched children without HbSS were recruited as controls; they were siblings of the SCD children or recruited from local schools. Two cohorts were tested, each on two occasions. In Cohort One, changes in lung function over a median follow-up of approximately two years and in Cohort Two [26] changes in lung function of children over a median follow up of ten years were determined. Cohort Two were recruited earlier during a previous period of research grant funding and both cohorts were followed during a recent period of grant funding. During this period, routine assessment of lung function of SCD patients was not undertaken. Only patients who successfully performed both spirometry and body plethysmography were included in the analysis. The study was approved by the King's College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

Lung function assessments

Patients were assessed in the Amanda Smith Pulmonary Function Laboratory at King's College Hospital NHS Foundation Trust. No subject underwent lung function testing within two weeks of an upper respiratory tract infection or within a month of suffering a vaso-occlusive crisis. A history was taken of past and current respiratory symptoms and medication for respiratory problems. Standing height was measured using a wall-mounted

stadiometer (Holtain ltd, Crymych, Dyfed, UK) and weight using electronic weighing scales (Seca ltd, Birmingham, UK).

Spirometry was performed and static lung volumes were assessed using whole-body plethysmography according to American Thoracic Society/European Thoracic Society criteria. Forced expiratory volume in one second (FEV_1), vital capacity (VC), forced expiratory flow between twenty-five and seventy-five % of VC (FEF_{25-75}), total lung capacity (TLC) and residual volume (RV) were recorded and the results expressed as the percent predicted for height, age and sex [29,30]. The highest forced vital capacity result obtained from spirometry and the slow vital capacity from the "plethysmography" manoeuvre was reported as VC. Subjects were assessed wearing a nose clip and breathing through a mouth piece. Measurements were performed using a pneumotachograph based system (Jaeger Masterscreen PFT, Carefusion Ltd, Basingstoke UK). Spirometry was repeated following administration of a bronchodilator (400µg salbutamol via a MDI and spacer) and a positive response was defined as an increase in FEV_1 of greater than or equal to 12% from baseline. Results were expressed as percent predicted for height, age, and sex using the ethnic-specific reference equations for spirometry [29] and the European Community for Steel and Coal Statement of the European Respiratory Society reference equations for lung volumes and gas transfer [31] for patients over eighteen years of age and those of Rosenthal et al for children under eighteen [30]. The predicted values for total lung capacity were reduced by 12% and residual volume by 7% to correct for ethnicity [32]. The lower and upper limits of normal were defined as the fifth and ninety-fifth percentiles respectively of the appropriate reference range. Patients were diagnosed with a restrictive abnormality if their TLC was less than the lower limit of normal (LLN) with a normal FEV_1:VC, an obstructive abnormality if their

FEV_1:VC was less than LLN and a mixed pattern if both TLC and FEV_1:VC were less than the LLN [32].

Clinical and laboratory characteristics

The medical records for all SCD children were examined. The occurrence and number of ACS episodes during the study period was recorded for each SCD child. An ACS episode was diagnosed if the child had suffered chest pain, dyspnoea and pyrexia together with a new pulmonary infiltrate on chest radiograph [19]. SCD children and controls were diagnosed as having asthma if they were currently prescribed any anti-asthma medications. Whether the child was on a chronic transfusion program or receiving hydroxurea was also noted. Hemoglobin concentrations for the SCD children were obtained from routine clinical blood samples taken within two months of lung function testing and when the patient was clinically stable.

Statistical analysis

Differences in lung function results at baseline and follow-up were assessed for statistical significance using the Wilcoxon signed-rank test, Fisher's exact test or Chi-squared test as appropriate. Individual lung function results were reported as the percentage predicted in order to normalise results for stature and where appropriate age. Linear mixed model (LMM) analysis was then used to analyse trajectories of decline in lung function in the SCD children relative to the control groups and to determine the influence of asthma, airways obstruction at baseline and the occurrence of ACS during the follow up period. ACS, airways obstruction at baseline and diagnosis of asthma were coded as nominal variables (0 = absent; 1 = present). Models were also fitted to compare the trajectories in the two cohorts. LMM

analysis is an extension of multiple regression, which allows irregularly spaced serial data for individuals to be combined in a single linear regression model, providing estimates of both individual changes over time and group patterns. Fixed effects in the models were: ACS during the study period, diagnosis of asthma, obstruction at baseline, age at measurement, and an intercept term. Random effect variables comprised age at measurement and an intercept term. Models were estimated using restricted maximum likelihood (REML), and separate models were fitted for each of the lung function results with all models grand-mean centred on age at baseline such that the intercept terms would be interpretable as the mean value of the result at baseline, and the slope would represent the annual rate of change. Interaction terms were introduced to test the equality of slopes between groups. Initial models included all variables and interactions. The validity of including the random effects, and the selection of an appropriate covariance structure for the residuals was assessed by likelihood ratio testing based on the -2 REML log-likelihood difference between the original and modified models. Models were then reduced by the sequential removal of non-significant terms. Likelihood ratio tests based on the difference in -2 ML log-likelihood for initial and reduced models were used to determine whether a non-significant term should be removed [34]. Baseline predictors of future ACS were determined using binary logistic regression.

RESULTS

Demographics

Cohort One: Forty-seven children with SCD and twenty-six controls were assessed; the two groups were of similar age and sex distribution (Table 1). Ten SCD children (21%) had had at least one ACS episode during the study. Nine SCD children and two controls had a diagnosis of asthma ($p = 0.308$). Seven SCD children (14.9%) were prescribed hydroxyurea and one (2.1%) was on a chronic transfusion programme within the study period. The majority of the SCD children patients (45) and controls (25) were aged five or above at baseline.

Cohort Two: Forty-five children with SCD and twenty-six controls were assessed; the two groups were of similar age and sex distribution (Table 1). Twelve SCD children (27%) had had at least one ACS episode during the study. Three SCD children and four controls had a diagnosis of asthma ($p = 0.227$). Eight SCD children (17.7%) were prescribed hydroxyurea and two (4.4%) was on a chronic transfusion programme within the study period. The majority of SCD children (43) and controls (24) were aged five or above.

The SCD patients in Cohort One were significantly younger at baseline compared to those in Cohort Two ($p = 0.007$) (Table 1). The age of the controls in the two cohorts did not differ significantly. The children in Cohort One had a mean of 0.58 (range 0-8.7) ACS episodes/year and Cohort Two a mean of 0.09 (range 0-1.3) ACS episodes ($p=0.0355$). If only the children who had at least one ACS episode were considered in Cohort One this was a median of 0.65 (range 0.38-8.7) episodes/year and in Cohort Two 0.11 (range 0.08-1.26)

episodes/year ($p<0.0001$). The only significant difference in lung function of the two SCD cohorts at recruitment was that Cohort One had a higher TLC ($p=0.0247$). There were no significant differences in the lung function results of the two control groups at recruitment.

Lung function changes with increasing age

In both cohorts, lung function declined significantly in the SCD children, but in neither control group (Tables 2 and 3). Lung function results as z-scores are presented in tables E1 and E2 in the online supplement.

Lung function abnormalities at baseline and follow-up

At baseline, sixteen SCD children in Cohort One (34%) had an obstructive pattern, and one (2%) a restrictive defect; no child had a mixed defect. At follow-up, the number of subjects with an obstructive defect had declined to five (10.6%, $p = 0.021$), the number with restrictive defects increased to eight (17%, $p = 0.034$) and two patients had a mixed pattern (4.3%, $p = 0.495$). A positive response to a bronchodilator was seen in four SCD children (16.7%) at baseline and three (12.5%) at follow-up ($p= 1.000$). In Cohort Two, at baseline, eleven SCD children (24%) had an obstructive pattern and five (11%) a restrictive defect; no child had a mixed defect. At follow-up, five subjects had an obstructive defect (11.1%, $p = 0.167$), the number with restrictive defects had increased to twenty (44%, $p = 0.0008$) and three patients had a mixed pattern (6.7%, $p = 0.242$). A positive bronchodilator response was seen in seven subjects (15.6%) at baseline and one subject (2.2%) at follow-up ($p=0.059$).

At baseline, the proportion of SCD children with obstructive and restrictive defects was similar in both cohorts ($p = 0.364$, $p = 0.107$ respectively). At follow-up, Cohort Two

compared to Cohort One had a greater proportion of SCD children with a restrictive defect (p = 0.006), but a similar number with an obstructive defect (p = 0.792). In Cohort Two, patients with an obstructive defect at baseline were more likely to have a restrictive abnormality at follow-up (p=0.0289).

In neither control group was there a significant change in the patterns of lung function over the follow up period. Amongst the controls in Cohort One, three had an obstructive abnormality and none a restrictive abnormality at baseline and follow up. Amongst the controls in Cohort Two, two had an obstructive lung function abnormality and none a restrictive abnormality at baseline and follow up.

Lung function abnormalities and ACS

In both cohorts, there were significant correlations in the SCD children between obstructive defects at baseline and ACS episodes (p=0.0003 for Cohort One, p=0.028 for Cohort Two). In Cohorts One and Two, in the SCD children the presence of obstructive defects at baseline was predictive of an ACS episode during the study: odds ratio (OR) 13.9 (95%CI 2.5 – 77.0), p= 0.003 and 5.4 (95%CI 1.2 – 23.7), p= 0.026 respectively. In Cohort One there was no independent association in the SCD children between asthma and a subsequent ACS episode: OR 6.1 (95% CI -0.5 – 12.8, p = 0.070). ACS episodes occurred more frequently in the SCD children in Cohort One than Cohort Two (one episode per 1.93 patient/years versus one episode per 12.6 patient/years (p<0.0001).

Lung function decline in SCD children and controls

In Cohort One, a significant decline was observed in the SCD children relative to the controls in FEV_1 (p=0.027), VC (p<0.0001), FEF_{25-75} (p=0.042), TLC (p<0.0001) and RV (p=0.001) (see supplement Table E3) . In Cohort Two, a significant decline was observed relative to the controls in FEV_1 (p=0.001), VC (p=0.004), FEF_{25-75} (p=0.016) and TLC (p=0.002) (see supplement Table E4) . The rate of decline was significantly greater in Cohort One than Cohort Two for FEV_1 (p = 0.008), VC (p = 0.001), FEF_{25-75} (p = 0.030), TLC (p = 0.004), and RV (p = 0.043) (see supplement Table E5).

Predictors of lung function decline in SCD children

In Cohort One, obstructive defects at baseline were linked to a lower intercept (baseline value) for FEV_1 (p = 0.027) and FEV_1:VC (p < 0.0001). The occurrence of ACS episodes was associated with a more rapid decline (i.e. greater negative slope) for FEV_1 (p=0.005), VC (p=0.022), FEF_{25-75} (p=0.003) and TLC (p=0.004). In Cohort Two, obstructive defects at baseline were linked to a lower intercept (baseline value) for FEV_1 (p = 0.011), FEV_1:VC (p < 0.0001), and FEF_{25-75} (p = 0.013), whilst ACS episodes were associated with a more rapid decline (i.e.: greater negative slope) for VC (p = 0.031) and TLC (p = 0.026), and an increase (i.e. more positive slope) in FEV_1:VC (p = 0.049) (see supplement Tables E6 and E7). No significant effects of hydroxyurea use or chronic transfusion were seen during exploratory analysis when those factors were included as covariates in the models (see supplement Tables E6 and E7), nor did their inclusion as non-significant terms improve the model fit as assessed by likelihood ratio testing.

DISCUSSION

We have demonstrated that lung function declines in children with SCD compared to similar aged, ethnic matched controls. The average annual rate of decline for all lung volumes was significantly greater in Cohort One compared to Cohort Two, which suggests the most rapid period of deterioration takes place during early childhood. This deterioration represents a progression towards a restrictive pattern of lung function with increasing age. We demonstrated that ACS episodes were the only independent predictor of a greater decline in lung volumes. ACS episodes occurred more frequently per unit follow up period in Cohort One who were followed up for two years compared to Cohort Two who were followed up for ten years. Thus ACS episodes might explain the greater rate of decline in lung function in Cohort One and hence our results emphasize the need for more effective methods of preventing ACS episodes, such as an increased use of hydroxyurea.

The rate of decline in lung volumes observed in the SCD children in Cohort One, that is the younger children, was similar to that demonstrated by Maclean et al [17], who observed an annual reduction in TLC of around 2.3% per year, with proportionate reductions in FEV_1, VC, and FEF_{25-75}. Unlike Maclean et al, however, we did not detect a decline in RV:TLC in the SCD children. This difference may be explained by Maclean not expressing RV:TLC as a percentage of the predicted value and therefore not accounting for age-related changes in RV:TLC [35].

The prevalence of obstructive lung disease in the SCD children at baseline was similar in both cohorts (34 and 24% respectively) and to that observed by Koumbourlis et al (22%)

[16], but higher than that reported by MacLean et al. The latter difference may be due to MacLean's use of a fixed cutoff for FEV_1:VC to define an obstructive defect, whereas this study used the lower limit of normal based on a reference range. After criticism from Koumbourlis [36], Maclean and coworkers subsequently reanalyzed their data using the lower limit of normal to define abnormality and found that more patients had a diagnosis of obstruction than reported in the original study [37]. The proportion of patients with restrictive disease at baseline was lower in the SCD children Cohort One to that observed by Koumbourlis (22%), but similar in Cohort Two. Cohort One was, however, younger than the cohort of Koumbourlis (8.8 versus 10.6 years), whereas Cohort Two was of a similar age (10.2 years) [16]. The prevalence of restrictive defects at baseline was higher at follow-up in Cohort Two than in Maclean's cohort when assessed at age seventeen (18.7%) [17]. That difference may be explained by Maclean using a fixed cutoff of <70% predicted for TLC to define restriction, which may have resulted in mis-diagnosis.

Although ACS episodes were linked to a faster progression of restrictive disease, whether the prompt treatment of airflow obstruction in SCD might help prevent the development of restrictive lung disease in later life is unknown. The incidence of obstructive defects at baseline was higher than that of a diagnosis of asthma, which suggests other mechanisms may be responsible for obstructive defects in SCD. Indeed, we have recently demonstrated an association between pulmonary vascular engorgement and obstructive lung function in both adults [38] and children with SCD [15]. Hence, any preventative strategy needs to consider this abnormality in SCD children.

This study has strengths and some limitations. The novelty of our data is that we have longitudinally assessed SCD patients and ethnic and age matched controls. .To the best of our knowledge, this is the first longitudinal study to include matched controls evaluated in parallel with SCD children. We also monitored patients prospectively and used a consistent definition to document the occurrence of ACS episodes during the study period. The inclusion of two SCD groups with two and ten-year follow-up periods respectively allowed both the short and long term changes in lung function in childhood to be evaluated. We used ethnic-specific reference equations for spirometric results, but the static lung volume results were related to two reference ranges derived from Caucasian children and adults with a fixed correction factor to account for ethnicity as no ethnic-specific reference values are currently available. This adjustment is, however, imprecise and may have resulted in some inaccuracy. The change in lung function was, therefore, referenced to an ethnic-matched control group in each cohort so that interpretation of within-cohort changes in the SCD children was valid. Indeed, the pattern of change in static lung volumes was similar to that in spirometric measurements. We did not exclude patients who were treated with hydroxyurea or chronic transfusions, as this would have biased the study population in favor of SCD children with less severe disease. Inclusion of those treatments into the analysis did not result in any significant differences in the results, but the number of children receiving such treatments was small and hence it would be inappropriate to draw conclusions from those results.

In conclusion, lung function in children with sickle cell disease declines and the rate of decline was greater in young children in whom ACS episodes were more common. Our results suggest treatment strategies to prevent ACS episodes need to be started in young SCD children if they are to be most effective in preventing the decline in lung function.

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Authors contributions: AG and AL designed the study, AL, KS, and EMcG collected the data and AL and AG analysed the data. All authors were involved in the production of the manuscript .

REFERENCES

1. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, Temperley WH, Williams TN, Weatherall DJ, Hay SI. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. The Lancet 2013;381:142-151.
2. Quinn CT, Rogers ZR, McCavit TL, Buchanan GR. Improved survival of children and adolescents with sickle cell disease. Blood 2010;115:3447-3452.
3. Parent F, Bachir D, Inamo J, Lionnet F, Driss F, Loko G, Habibi A, Bennani S, Savale L, Adnot S, Maitre B, Yaïci A, Hajji L, O'Callaghan DS, Clerson P, Girot R, Galacteros F, Simonneau G. A hemodynamic study of pulmonary hypertension in sickle cell disease. N Engl J Med 2011;365:44-53.
4. Powars D, Weidman JA, Odom-Maryon T, Niland JC, Johnson C. Sickle cell chronic lung disease: prior morbidity and the risk of pulmonary failure. Medicine (Baltimore), 1988;67:66-76.
5. Klings ES, Wyszynski DF, Nolan VG, Steinberg MH. Abnormal pulmonary function in adults with sickle cell anemia. Am J Respir Crit Care Med 2006;173:1264-1269.
6. Delclaux C, Zerah-Lancner F, Bachir D, Habibi A, Monin JL, Godeau B, Galacteros F. Factors associated with dyspnea in adult patients with sickle cell disease. Chest 2005;128:3336-3344.
7. Santoli F, Zerah F, Vasile N, Bachir D, Galacteros F, Atlan G. Pulmonary function in sickle cell disease with or without acute chest syndrome. Eur Respir J 1998;12:1124-1129.
8. Girgis RE, Qureshi MA, Abrams J, Swerdlow P. Decreased exhaled nitric oxide in sickle cell disease: relationship with chronic lung involvement. Am J Hematol 2003;72:177-184.

9. Sylvester KP, Desai SR, Wells AU, Hansell DM, Awogbade M, Thein SL, Greenough A. Computed tomography and pulmonary function abnormalities in sickle cell disease. Eur Respir J 2006;28:832-838.

10. Pianosi P, D'Souza SJ, Charge TD, Esseltine DE, Coates AL. Pulmonary function abnormalities in childhood sickle cell disease. J Pediatr 1993;122:366-371.

11. Sylvester KP, Patey RA, Milligan P, Dick M, Rafferty GF, Rees D, Thein SL, Greenough A. Pulmonary function abnormalities in children with sickle cell disease. Thorax 2004;59:67-70.

12. Koumbourlis AC, Zar HJ, Hurlet-Jensen A, Goldberg MR. Prevalence and reversibility of lower airway obstruction in children with sickle cell disease. J Pediatr 2001;138:188-192.

13. Koumbourlis AC, Hurlet-Jensen A, Bye MR. Lung function in infants with sickle cell disease. Pediatr Pulmonol 1997;24:277-281.

14. Chaudry RA, Rosenthal M, Bush A, Crowley S. Reduced forced expiratory flow but not increased exhaled nitric oxide or airway responsiveness to methacholine characterises paediatric sickle cell airway disease. Thorax 2014;69:580-585.

15. Wedderburn CJ, Rees D, Height S, Dick M, Rafferty GF, Lunt A, Greenough A. Airways obstruction and pulmonary capillary blood volume in children with sickle cell disease. Pediatr Pulmonol 2014;49:716-722.

16. Koumbourlis AC, Lee DJ, Lee A. Longitudinal changes in lung function and somatic growth in children with sickle cell disease. Pediatr Pulmonol 2007;42:483-488.

17. MacLean JE, Atenafu E, Kirby-Allen M, MacLusky IB, Stephens D, Grasemann H, Subbarao P. Longitudinal decline in lung volume in a population of children with sickle cell disease. Am J Respir Crit Care Med 2008;178:1055-1059.

18. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994;330:1639-1644.

19. Vichinsky EP, Neumayr LD, Earles AN, Williams R, Lennette ET, Dean D, Nickerson B, Orringer E, McKie V, Bellevue R, Daeschner C, Manci EA. Causes and outcomes of the acute chest syndrome in sickle cell disease. National Acute Chest Syndrome Study Group. N Engl J Med 2000;342:1855-1865.

20. Knight-Madden JM, Forrester TS, Lewis NA, Greenough A. The impact of recurrent acute chest syndrome on the lung function of young adults with sickle cell disease. Lung 2010;188:499-504.

21. Knight-Madden JM, Forrester TS, Lewis NA, Greenough A. Asthma in children with sickle cell disease and its association with acute chest syndrome. Thorax 2005;60:206-210.

22. Sylvester KP, Patey RA, Milligan P, Rafferty GF, Broughton S, Rees D, Thein SL, Greenough A. Impact of acute chest syndrome on lung function of children with sickle cell disease. J Pediatr 2006;149:17-22.

23. Boyd JH, Macklin EA, Strunk RC, DeBaun MR. Asthma is associated with acute chest syndrome and pain in children with sickle cell anemia. Blood 2006;108:2923-2927.

24. Bryant R. Asthma in the pediatric sickle cell patient with acute chest syndrome. J Pediatr Health Care 2005;19:157-162.

25. Nordness ME, Lynn J, Zacharisen MC, Scott PJ, Kelly KJ. Asthma is a risk factor for acute chest syndrome and cerebral vascular accidents in children with sickle cell disease. Clin Mol Allergy 2005;3:2.

26. Sylvester KP, Patey RA, Broughton S, Rafferty GF, Rees D, Thein SL, Greenough A. Temporal relationship of asthma to acute chest syndrome in sickle cell disease. Pediatr Pulmonol 2007;42:103-106.

27. Intzes S, Kalpatthi RV, Short R, Imran H. Pulmonary function abnormalities and asthma are prevalent in children with sickle cell disease and are associated with acute chest syndrome. Pediatr Hematol Oncol 2013;30:726-732.

28. Castro O, Brambilla DJ, Thorington B, Reindorf CA, Scott RB, Gillette P, Vera JC, Levy PS. The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. Blood 1994;84:643-649.

29. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95 year age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-1343.

30. Rosenthal M, Cramer D, Bain SH, Denison D, Bush A, Warner JO. Lung function in white children aged 4 to 19 years: II--Single breath analysis and plethysmography. Thorax 1993;48:803-808.

31. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:5-40.

32. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-968.

33. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J 2005;26:319-338.

34. West BT. Analyzing longitudinal data with the linear mixed models procedure in SPSS. Eval Health Prof 2009;32:207-208.

35. Quanjer PH, Stanojevic S, Stocks J, Hall GL, Prasad KV, Cole TJ, Rosenthal M, Perez-Padilla R, Hankinson JL, Falaschetti E, Golshan M, Brunekreef B, Al-Rawas O, Kühr J, Trabelsi Y, Ip MS; Global Lungs Initiative. Changes in the FEV1/FVC ratio during childhood and adolescence: an intercontinental study. Eur Respir J 2010;36:1391-1399.

36. Koumbourlis AC, Lee DJ, Lee A. Changes in lung function in children with sickle cell disease. Am J Respir Crit Care Med 2009;180:377.

37. MacLean JE, Grasemann H, Subbarao P. Changes in Lung Function in Children with Sickle Cell Disease. Am J Respir Crit Care Med 2009;180:377-378.

38. Lunt A, Desai SR, Wells AU, Hansell DM, Mushemi S, Melikian N, Shah AM, Thein SL, Greenough A. Pulmonary function, CT and echocardiographic abnormalities in sickle cell disease. Thorax 2014;69:746-751.

Table 1: Demographics by SCD status

Data are demonstrated as n (%) or median (range)

	SCD	**Controls**	**p**
Cohort One			
N	47	26	
Males (%)	21 (45%)	7 (27%)	0.352
Age at baseline (years)	8.8 (3.0-13.1)	10.2 (4.0-14.6)	0.072
Time to follow-up (years)	2.0 (0.9-3.5)	1.7 (1.5-2.51)	0.518
Hb (g/dL)	8.2 (4.6-11.9)		
Cohort Two			
N	45	24	
Males (%)	19 (42.2%)	8 (33.3%)	0.81
Age at baseline (years)	10.2 (4.3-16.0)	8.5 (4.0-17.8)	0.24
Time to follow-up (years)	9.9 (6.0-13.5)	11.4 (7.0-13.4)	0.063
Hb (g/dL)	8.2 (5.7-12.8)		

Table 2: Lung function in Cohort One by follow up status.

Data are presented as expressed as percent predicted and median (range) and.

	Baseline	**Follow-up**	**p**
SCD children			
FEV_1	91.6 (70.5-117.5)	88.7 (55.5-122.6)	0.0297
VC	97.2 (67.3-140.8)	91.8 (59.4-123.5)	0.0002
FEF_{25-75}	91.8 (40.4-189.6)	82.4 (29.7-146.7)	0.0001
FEV_1/VC	94.7 (71.8-109.8)	95 (72.2-111.8)	0.2318
TLC	97.2 (72.1-127.1)	89.7 (68.5-121.4)	<0.0001
RV	107.4 (40.6-212.0)	95.5 (42.2-160.0)	0.0032
RV/TLC	128.2 (68.2-218.5)	120.1 (52.5-182.8)	0.469
Controls			
FEV1	94.2 (73.4-129.3)	100.6 (76.7-131.0)	0.2427
VC	98.9 (79.6 – 129.2)	99.2 (80.5 – 136.6)	0.0988
FEF25-75	96.3 (37.5-160.1)	96.7 (45.5-158.6)	0.3219
FEV1/VC	98.5 82.3-108.8)	97.3 (76.9-112.8)	0.5938
TLC	94.8 (80.8-115.5)	98.2 (78.2-115.5)	0.8789
RV	101.4 (66.6-172.2)	102.5 (57.2-161.6)	0.3539
RV/TLC	104.7 (72.1-137.1)	100.2 (62.2-160.8)	0.4164

Table 3: Lung function in Cohort Two by follow up status.

Data are expressed as percent predicted and presented as median (range).

SCD children	**Baseline**	**Follow-up**	**p**
FEV_1	90.7 (64.0-117.2)	81.2 (66.4-106.7)	0.0002
VC	97.6 (62.6-116.7)	85.4 (68.7-109.6)	0.0003
FEF_{25-75}	91.8 (45.9-144.9)	74.5 (28.9-122.7)	<0.0001
FEV_1/VC	96.2 (69.7-109.4)	95.4 (64.4-108.1)	0.7648
TLC	92.5 (67.6-127.1)	81.6 (61.0-108.3)	<0.0001
RV	101.2 (37.7-212.0)	88.9 (54.8-149.2)	0.0300
RV/TLC	121.2 (73.4-194.3)	113.2 (67.5-221.5)	0.0692
Controls			
FEV1	99.8 (73.8-128.8)	100.3 (76.8-148.7)	0.0946
VC	103.1 (71.3-131.2)	102.7 (86.2-124.6)	0.1747
FEF25-75	92.7 (55.3-168.3)	102.3 (54.7-163.0)	0.0891
FEV1/VC	98.8 (81.1-112.2)	96.3 (84.9-116.6)	0.7425
TLC	98.3 (78.5-111.8)	94.0 (84.2-121.8)	0.9421
RV	95.7 (50.5-165.1)	91.1 (33.0-154.3)	0.9431
RV/TLC	110.2 (63.2-175.7)	94.6 (45.0-158.7)	0.0742